Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy

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Summary

Background Early assessment of antiretroviral drug efficacy is important for prevention of the emergence of drug-resistant virus and unnecessary exposure to ineffective drug regimens. Current US guidelines for changing therapy are based on measurements of plasma HIV-1 RNA concentrations 4 or 8 weeks after the start of treatment with cut-off points of 0·75 or 1·00 log, respectively. We investigated the possibility of assessing drug efficacy from measurements of plasma HIV-1 concentrations made during the first week on therapy.

Methods The kinetics of virus decay in plasma during the first 12 weeks of treatment was analysed for 124 HIV-1-infected patients being treated for the first time with a protease inhibitor. Patients with a continuous decline of HIV-1 concentrations and in whom HIV-1 was either undetectable or declined by more than 1·5 log at 12 weeks were defined as good responders; the rest were poor responders.

Findings The individual virus decay rate constants (k) at day 6 correlated significantly (r>0·66, p<0·0001) with changes in HIV-1 concentrations at 4, 8, and 12 weeks, and correctly predicted 84% of the responses with a cut-off value of k=0·21 per day (in log scale). Reduction in plasma HIV-1 of less than 0·72 log by day 6 after initiation of therapy predicted poor long-term responses in more than 99% of patients.

Interpretation These results suggest that changes in HIV-1 concentration at day 6 after treatment initiation are major correlates of longer-term virological responses. They offer a very early measure of individual long-term responses, suggesting that treatment could be optimised after only a few days of therapy.

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Introduction

Concentrations of HIV-1 RNA in plasma predict the risk of AIDS and death during the natural course of the disease,[1] and act as a marker of the efficacy of antiretroviral therapy.[2–5] To assess virological responses to therapy, samples of plasma are typically assayed for HIV-1 RNA 4–12 weeks after the start of treatment; the kinetics of the initial response to therapy is generally not measured. According to guidelines produced by the US Department of Health and Human Services, specific criteria that should prompt consideration for changing therapy include less than a 0·50–0·75 log reduction in plasma HIV-1 RNA by 4 weeks after the start of therapy, or less than a 1·00 log reduction by 8 weeks.[6] Evaluation of changes in virus load 4–12 weeks after the start of treatment can serve as a prognostic indicator for longer-term virological responses.[7–9] However, viral resistance can develop during this time if the therapy is suboptimal, and patients could be unnecessarily exposed to ineffective and toxic drugs. Therefore prediction of drug efficacy on the basis of data obtained during the first few days of treatment could be helpful for optimising therapy.

We postulated that the initial slopes of HIV-1 concentration changes during the first week of treatment depend on drug efficacy and can be used as an early predictor of longer-term response to antiretroviral treatment. Previous results for a group of paediatric patients who varied in their responses to ritonavir monotherapy[10] indicated that some of the patients received suboptimal or failing therapy, which allowed us to investigate factors predictive of drug efficacy. Changes in plasma HIV-1 RNA concentration during the first week on therapy, drug concentrations in plasma at the end of the first week on therapy, and baseline HIV-1 RNA concentrations and CD4 cell counts, but not patients' age, were predictors of virological response to treatment at 12 weeks on therapy.[10] Because the changes in HIV-1 RNA concentrations at 12 weeks are, in turn, predictive of longer-term virological and immunological responses, we argued that early measurements of HIV-1 dynamics could also be predictive of responses at times longer than 12 weeks.

Wu and colleagues[11] and Mittler and colleagues[12] also analysed the initial rate of virus decline as a predictor of drug efficacy. Wu and colleagues found that viral decay rates are not predictive of treatment failure. However, the treatment was changed in that study from ritonavir monotherapy to combination therapy at day 10. Mittler and colleagues assessed the efficacy of nelfinavir monotherapy, and showed a correlation between the rates of plasma HIV-1 RNA decline over the first 14–21 days and virus reduction by day 56. In both studies, sampling was not done on a daily basis during the first week on therapy, and the study design did not include measurements of initial virus decline for patients on highly active antiretroviral therapy (HAART). We assessed the possibility of using the very

early dynamics of HIV-1, assessed by measurement of daily samples for different cohorts of patients, including patients on HAART, as a possible predictor of drug efficacy.

Methods

Patients

Data were obtained from three cohorts of patients. The first cohort consisted of 52 children who had never received protease inhibitors, who completed 12 weeks of a phase I trial of indinavir monotherapy in 1996, and who received doses of either 250, 350, or 500 mg/m^2 indinavir every 8 h.[13] The complete indinavir study group consisted of 54 children. The second cohort comprised 34 adults, naïve to protease inhibitors and non-nucleoside reverse transcriptase inhibitors, who were treated with a four-drug combination regimen (zidovudine 300 mg twice daily, lamivudine 150 mg twice daily, nevirapine 200 mg once daily for 14 days then 200 mg twice daily, and indinavir 800 mg three times daily) during 1997–2000. We included all of the first 34 patients in the ongoing four-drug study. For these two cohorts of patients, blood samples were obtained every day during the first 6 days on therapy, and then at weeks 2, 4, 8, and 12 for the indinavir-treated patients, and at days 9, 12, 15, 19, 23, 27, 41, 62, and 82 for patients in the four-drug study. The third cohort consisted of 38 children, naïve to protease inhibitors, who completed 12 weeks of a phase I trial of ritonavir monotherapy in 1995–96 and who received doses of either 250, 300, 350, or 400 mg/m^2 ritonavir every 12 h.[10,14] The original ritonavir study group consisted of 48 children. Samples from the children in the third cohort were obtained at days 0 (the day therapy was started and before the morning drug dose), 1, 2, 3, and 6, and then at weeks 2, 4, 8, and 12 after the start of therapy.

The phase I trials of ritonavir and indinavir were done under clinical trial agreement between the US National Cancer Institute and Abbot Laboratories and Merck and Company, respectively. The three clinical trials were approved by the institutional review boards of the US National Cancer Institute (paediatric cohorts) or the US National Institute of Allergy and Infectious Diseases (adult cohort). For the three cohorts, only patients without measurements of samples obtained at day 6 or week 12 were excluded from the analyses.

Procedures

The number of CD4 cells was measured by flow cytometry. HIV-1 RNA concentrations in plasma were measured by PCR (Amplicor, Roche Diagnostic Systems, Branchburg, NJ, USA), the lower limit of detection of which was 200 copies per mL for ritonavir and indinavir monotherapy and 50 copies per mL for HAART. The baseline HIV-1 RNA concentrations were calculated as an average of two log concentrations from samples taken on day 0 and the previous day or a few days before. The baseline variance was smaller than 0·3 log. Good responders were defined as patients whose viraemia measurements after day 2 decreased continuously without a subsequent increase (except for fluctuations of less than 0·5 log between two descending concentrations) during the first 3 months on therapy, and those who had HIV-1 concentration changes greater than 1·5 log or undetectable HIV-1 at week 12. The rest of the patients were designated as poor responders.

Statistical analysis

The kinetics of plasma HIV-1 RNA during the first week after the start of therapy was analysed by a simple one-exponential model:

$$\log(V)=c-kt$$

where V=HIV-1 RNA concentration, k=virus decay rate constant in log scale, and c=constant close to the baseline virus concentration V_0. The relation of k to decay rate constant in ln scale k_l is: $k_l=k\ln 10=2{\cdot}3k$. The least squares approach was used for data fitting to the model. The data fitting and goodness-of-fit analysis were done with the program Scientist (MicroMath, Salt Lake City, UT, USA).

For prediction, we used logistic regression analysis with a binary outcome of poor and good response. Multiple logistic regression analysis was used for evaluation of the effects of baseline HIV-1 and CD4 T-cell counts. The program Statistica 4.5 (Statsoft, Tulsa, OK, USA) was used for these and basic statistical analyses. The robustness of the prediction was tested by cross-validation procedures based on the jack-knife technique.[15] Data for each patient were removed sequentially from each cohort of patients and from the three cohorts combined, and the logistic regression equation coefficients were calculated and used for new prediction.

The robustness of the prognosis was assessed by the number of incorrect predictions and by the reduction of the correlation between calculated and observed type of response after the application of the cross-validation techniques. The specificity of the decay rate constant as a predictor was assessed by comparison of the correlation coefficients between observed and calculated responses by use of parameters with low predictive power (age) as negative controls. The statistical significance of the prediction was determined by permutation tests based on randomisation of the types of responses for all patients. These stochastic responses were used for prediction. The correlation coefficient between the predicted and the stochastic responses was calculated for 15 randomised sets of responses and the likelihood of correct prognosis occurring by chance was assessed.

Results

There was no significant difference in the baseline plasma HIV-1 RNA concentration between groups on monotherapy (ritonavir or indinavir) and on HAART ($p>0{\cdot}21$, table). The combined group of patients on monotherapy had about the same baseline HIV-1 RNA concentration (4·91) as those on the four-drug study (4·95, $p=0{\cdot}81$).

The mean change in log HIV-1 RNA concentration for poor and good responders was 0·82 and 2·10 log, respectively, at week 4, and 0·50 and 2·60 log at week 8 (figure 1). All patients with less than a 0·75 log or 1·00 log decrease in viral loads at week 4 or 8, respectively, were poor responders. Most patients treated with indinavir monotherapy (48 of 52) responded poorly; by contrast, all HAART patients were good responders (table). The calculated average rate constant of HIV-1 decline during the first 6 days on therapy for the HAART patients was slightly greater than the average rate constants for the good responders treated with ritonavir ($p=0{\cdot}34$) or indinavir ($p=0{\cdot}08$; table). This finding suggests that good responders from different cohorts behave similarly with respect to HIV-1 dynamics

	Good responders				Poor responders				All patients			
	n	Mean (SD) log HIV_0	Mean (SD) $CD4_0$ (cells/μL)	Mean (SD) *k*	n	Mean (SD) log HIV_0	Mean (SD) $CD4_0$ (cells/μL)	Mean (SD) *k*	n	Mean (SD) log HIV_0	Mean (SD) $CD4_0$ (cells/μL)	Mean (SD) *k*
Indinavir	4	5·20 (0·32)	683 (737)	0·21 (0·07)	48	4·71 (0·76)	243 (266)	0·08 (0·07)	52	4·75 (0·74)	277 (333)	0·09 (0·08)
Ritonavir	13	4·76 (0·79)	493 (556)	0·24 (0·09)	25	5·33 (0·49)	183 (339)	0·16 (0·09)	38	5·14 (0·66)	289 (444)	0·18 (0·09)
HAART	34	4·95 (0·62)	332 (225)	0·26 (0·05)	0	..	..	..	34	4·95 (0·62)	332 (225)	0·26 (0·05)
All treatments	51	4·92 (0·65)	401 (390)	0·25 (0·06)	73	4·92 (0·74)	223 (292)	0·10 (0·08)	124	4·92 (0·70)	296 (345)	0·17 (0·10)

HIV_0=HIV-1 RNA concentration at baseline. $CD4_0$=CD4 counts at baseline. Levels of statistical significance for differences between good and poor responders on ritonavir, indinavir, and all patients were respectively: p=0·01, 0·001, and <0·0001 (*k*); p=0·01, 0·21, and 1·00 (baseline HIV-1); p=0·04, 0·01, and 0·04 (baseline CD4 T cell counts). Level of significant difference between good responders on HAART and ritonavir p=0·34 or indinavir p=0·08 for *k* (slope).

Numbers of good and poor responders, HIV-1 RNA concentration and CD4 counts at baseline, and rate constant of HIV-1 decline (*k*) during first 6 days on therapy

and justifies their combined use for prognosis of longer-term response to therapy. The average rate constant for the HAART patients was significantly greater than those for the whole cohorts of ritonavir patients (0·18 per day [SD 0·09]) and indinavir patients (0·09 per day [0·08], p<0·0001), and the corresponding subcohorts of poor responders (0·16 per day [0·09] and 0·08 per day [0·07], respectively; p<0·0001).

More than 95% of the patients with rate constants of less than 0·16 per day had a poor response, and more than 95% of those with rate constants of greater than 0·28 per day had a good response (figure 2). This finding suggests that the likelihood for poor or good response is very high (>95%) for changes in the HIV-1 concentration of less than 0·96 log or higher than 1·68 log during the first 6 days on therapy. The cutoff value of *k*=0·21 per day approximately separated poor and good responses with a sensitivity of 0·85, a specificity of 0·82, and a predictive value of 0·87. The distribution of poor and good responders was approximately normal (mean 0·094 [SD 0·074] for poor responders and 0·250 [0·055] for good responders; figure 2). By using fitted normal distributions, we found that patients with a *k* value of less than 0·12 per day by day 6 (<0·72 log HIV-1 concentration changes) will be poor responders, with a probability of greater than 0·99.

The HIV-1 decay rate constants calculated from samples obtained during the first 3 (but not 1 or 2) days on therapy were also significantly different (p<0·0001) between good and poor responders (data not shown). However, the log change in the HIV-1 concentration by day 3 for all poor responders was similar to the assay accuracy (about two-fold, corresponding to 0·3 log), and although prediction is possible, it would not be as accurate as the one based on HIV-1 RNA concentrations at day 6. Rate constants calculated by using data for days 1–6 as well as by a model including the shoulder phase of the response time dependence yielded similar results to those for days 0–6 (data not shown). Several other combinations of starting and ending times were tested for calculation of the rate constants, but all had lower power of prediction. This finding suggests that the HIV-1 dynamics during the first 3–6 days on therapy are highly predictive of longer-term (12-week) response and correlate with drug efficacy.

A simpler method for calculation of the decline in HIV-1 RNA concentration yielded similar results. Subtraction of the log values of HIV-1 at day 6 from the ones at day 0, followed by division by 6 resulted in rate constants (k_{0-6}) with about the same values as the ones calculated by linear regression analysis (*k*), although the correlation with the type of response was lower (*r*=0·61 *vs* *r*=0·70, p=0·22). Thus, measurement of the virus load at study entry and day 6 of treatment can provide a simple method for assessment of drug efficacy. A logistic regression analysis suggested that the longer-term response can be predicted for 84% of all patients on the basis of their initial rate constants for days 0–6. A lower percentage (77%) of correctly predicted responses was found when using rate constants, k_{0-6}, based on single values of HIV-1 RNA concentration at days 0 and 6.

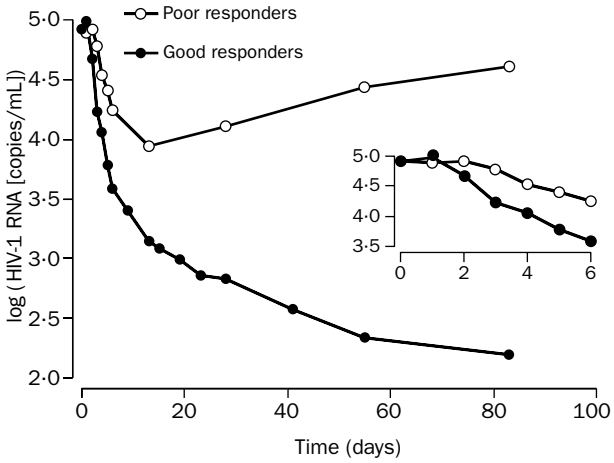

Figure 1: **Geometric mean values of plasma HIV-1 RNA concentration for poor and good responders**
Inset: HIV-1 dynamics during first 6 days after start of treatment.

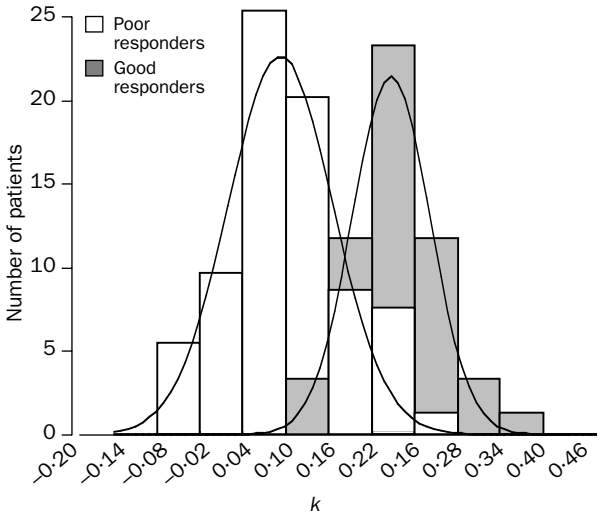

Figure 2: **Distributions of poor and good responders as a function of initial rate constants (*k*)**
Number of patients represented by each bar corresponds to the range of rate constants defined by the values on either side of the bar. Superimposed normal distribution curves are fitted from data derived from subcohorts of good and poor responders.

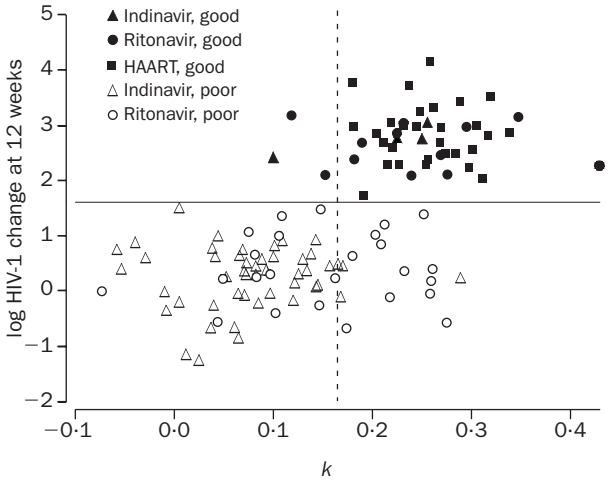

Figure 3: **Changes in HIV-1 concentration 12 weeks after start of treatment at different rate constants (*k*)**
Vertical line indicates 95% likelihood of poor response for $k<0{\cdot}16$.

The prediction was stable, as assessed by the jack-knife procedure: removal of each individual patient or all possible pairs of patients from the analysis did not affect the predictive power (mean correct prediction was 84·4% [SD 0·7, range 83·7–86·2] for individual patients and 84·5% [0·8, 83·6–86·9] for pairs of patients). It was also significantly different ($p<0{\cdot}0001$) compared with predictions based on randomised responses, as estimated by permutation tests (correlation between observed and predicted data $r=0{\cdot}87$ *vs* average $r=0{\cdot}30$ for the correlation between randomised responses and their prediction by the same method).

The coefficients of the logistic regression derived only from the ritonavir and ritonavir plus indinavir cohorts predicted correctly 75% and 76% of the responses of all patients, respectively. The baseline virus concentration and CD4 T-cell counts contributed to the prediction (85% correctly predicted responses), but for these cohorts of patients, their contribution was much smaller than the one due to virus decay rate constants. Age was not a predictor of long-term response (data not shown). Similar results were obtained with linear regression, and cluster and discriminant analyses.

The initial rate constants were predictive of longer-term response, and correlated highly with the changes of the log HIV-1 concentration at 2, 4, 8, and 12 weeks after the start of treatment relative to the baseline concentrations (correlation coefficients $r=0{\cdot}68$, 0·70, 0·67, and 0·66, respectively, $p<0{\cdot}0001$). An example of the dependence of HIV concentration changes at 12 weeks on the rate constants is shown in figure 3. However, the initial rate constants for poor responders did not correlate ($r=0{\cdot}086$, $p=0{\cdot}50$) with longer-term (12-week) log HIV-1 changes, suggesting stochastic mechanism of viral rebound in those patients or other mechanisms not related to the initial virus dynamics.

Discussion

We analysed HIV-1 dynamics during the first week on antiretroviral therapy in an attempt to assess longer-term virological responses. In doing so, we took advantage of what are now judged to be suboptimal regimens of ritonavir and indinavir monotherapy that were given several years ago as a part of phase I studies. For patients on single or combination drug therapy, the earliest and most important predictor of drug efficacy determining the longer-term (12-week) response was the change in the plasma HIV-1 RNA concentration at days 3–6 after the start of antiretroviral treatment. The likelihood of a poor response was more than 95% or 99% for patients with k constants of less than 0·16 or 0·12 per day, respectively, which corresponds to a decrease in HIV-1 RNA of less than 0·96 or 0·72 log, respectively, after 6 days on therapy. For such patients, therapy has a very high likelihood of failing or of being suboptimal and should be changed. With the current data, use of less extreme values of k would have less predictive value. The current specific criteria that now prompt consideration for changing therapy include less than a 0·50–0·75 log reduction in plasma HIV-1 RNA by 4 weeks after the start of therapy, or less than a 1·00 log reduction by 8 weeks.[6] Our results suggest that a much earlier assessment of drug efficacy might be feasible. Indeed, they suggest that a reduction in virus concentration of less than about 0·60 log by 6 days after the start of therapy is associated with a poor outcome in all patients at 12 weeks.

The rate constants of the decrease in HIV-1 concentrations calculated from multiple time points were better predictors than those calculated from the baseline level and a single value at day 6. However, the improvement obtained from the use of these additional points was not very great, suggesting that measurement of a single HIV-1 concentration on one day soon after the start of therapy can allow assessment of drug efficacy. A major problem for accurate prediction based on measuring a single value is the fluctuation in the virus concentration and the assay error. Thus, for statistical purposes, at least another intermediate time point at day 3, 4, or 5 is preferable.

Extending the times at which samples were taken to beyond day 6 (day 13 and 28) leads to poorer prediction by rate constants calculated by linear dependences of log HIV-1 on time from multiple points compared with those from single values (data not shown). Analysis suggested that this finding is due to the more complex time dependence of the virus concentration beyond day 6. Functions more complex than a single exponential function must be used for data fitting.[12,16,17] This finding can explain some of the previous observations that rate constants calculated by use of data points obtained after the first week in the absence of multiple points during the first week are not significantly predictive of longer-term response.[9,18] The relative decline in plasma HIV-1 concentration after 1 month on therapy, however, is predictive of long-term response, which is in agreement with recent data for an unselected general population of HIV-1-infected patients.[19] In further agreement with this finding, Mittler and colleagues[12] showed that changes in HIV concentration between days 4 and 21 are predictive of longer-term (day 56) virological response by use of a simple method that allowed the calculation of the relative efficacy of monotherapy compared with that of HAART. The lack of correlation between viral load reduction at day 56 and the relative efficacy for the first 7 days of treatment in their study is probably due to the poor response of most (more than 24 of 30) of their study participants, which is in agreement with our finding of lack of correlation between the initial rate constants and longer-term virological response in poor responders ($r=0{\cdot}086$, $p=0{\cdot}50$). A likely explanation for these findings is the stochastic nature of the evolution of resistant mutants in poor responders, leading to variable reduction in viral load at times longer than a few weeks on therapy and lack of correlation with slopes calculated during the first week on therapy.

The initial rate constants were also inversely correlated with the time, t_{50}, needed to reach undetectable (<50 copies per mL) levels of virus (not shown). For good responders on HAART, t_{50} was correlated with the baseline virus load (r=0·61) in agreement with the results of a recent study by Rizzardi and colleagues.[20] In the same study, suppression of viraemia to below 50 copies HIV-1 RNA per mL was correlated with successful virological response for a cohort of 118 patients naïve to antiretroviral therapy.[20] For cohorts of patients on one, three, or five drugs, the initial first week slopes were significantly different: higher slopes corresponded to more drugs used.[17] This finding relates to the finding that, on average, combination therapy is more efficient than monotherapy. Thus initial slopes are probably essential early indicators of successful therapy in other cohorts of antiretroviral-naïve patients.

Previously, we found that the trough concentration of ritonavir in plasma at the end of the first week on therapy for patients on monotherapy, but not the dose, correlated with the rate constants for virus decline and was predictive of longer-term response.[10] Similar observations were reported by Hoetelmans and colleagues.[21] These findings suggested that the initial slopes of changes in virus concentration are related to the concentration of drug in plasma. Mathematical models have been developed that take into account the dependence of virus slopes on drug efficacy.[11,17,22–25] These models differ in their assumptions, but all are based on the premise that drugs cannot completely block virus replication, and the slopes reflect the extent of virus replication inhibition. Simultaneous measurement of all drug concentrations in HAART and correlation of their concentrations with long-term response is difficult. Also, there will be differences in the sensitivities to drugs used among the virus strains in different patients. Our results suggest that, although measurements of trough drug concentrations might be desirable, initial virus slopes alone are sufficient for relatively accurate prediction of longer-term response. In addition to the initial slopes of virus concentration decline, baseline HIV-1 concentrations and CD4 T-cell counts were also predictive of longer-term viral load response but at lower significance. Patients' age did not correlate with longer-term changes in HIV-1 concentration, indicating that response to antiretroviral therapy is similar in children and adults. Drug concentration in plasma can be a strong predictor of drug efficacy, but we did not have sufficient information for all cohorts of patients to analyse its effect on longer-term virological responses.

This analysis was done for patients who were naïve to the drugs used. The existence of drug-resistant mutants is likely to affect the rate of virus decline dependent on their initial concentration. Early viral decline data might not accurately predict longer-term response in regimens in which resistance can develop very quickly (such as non-nucleoside reverse transcriptase monotherapy). This situation might also have occurred in several patients on ritonavir monotherapy who had high initial slopes but poor responses (figure 3). However, for patients who had high levels of drug-resistant mutants at entry, the initial slopes would be lower and this analysis would predict that the treatment is not efficient and must be changed. Therefore, we postulate that this analysis can also be used for patients with drug-resistant mutants: in all cases of low rate constants, the therapy must be adjusted at the end of the first week if the patient is taking and absorbing the drugs appropriately and if there are tolerable alternatives. However, for greater slopes, the response can be good or poor depending on the fitness of the pre-existing drug-resistant mutant, and prediction cannot be made.

Lack of adherence to treatment is a major problem in current HIV-1 treatments, and the approach proposed in this study could be only used if additional information on the adherence to the treatment is provided. The applicability of these results to other cohorts of patients is an open question. The finding that regression equations derived for one cohort of patients can be used for prediction in other cohorts suggests that the cohorts we analysed are sufficiently heterogeneous and representative to apply to a wider range of treatment protocols and individuals. However, further prospective studies with larger homogeneous cohorts and cohorts of drug-experienced patients at other institutions are needed to define more accurately the limits of this analysis.

Contributors

Dimiter Dimitrov and Michael Polis had the original idea, designed the overall study, analysed the results, and wrote the paper. Igor Sidorov did the mathematical modelling and numerical tests, and contributed to the overall data. Michael Polis, Christian Yoder, and Julia Metcalf were responsible for the design, collection, and interpretation of the data for the patients in the four-drug cohort. Brigitta Mueller and Phillip Pizzo were responsible for the design, collection, and interpretation of the data for the patients on indinavir and ritonavir monotherapy. Shirley Jankelevich and Robert Yarchoan contributed to the interpretation of the data for the indinavir and ritonavir monotherapy patients and to the overall design of the study. Mariana Dimitrov contributed to the statistical analyses and data interpretation. All investigators were involved in preparing the report for publication and contributed to the final version of the paper.

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